QUINOPROTEIN ETHANOL DEHYDROGENASE FROM *PSEUDOMONAS*

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ABSTRACT

Dye-linked ethanol dehydrogenases from *Pseudomonas aeruginosa* ATCC 17 933 and *P. putida* ATCC 17 421 were purified to homogeneity and crystallized. The amino acid composition of the two enzymes is very similar and the number of the aromatic amino acid residues found per subunit are almost identical.

With respect to their catalytic and molecular properties both ethanol dehydrogenases are similar to the quinoprotein methanol dehydrogenases known from methylotrophic bacteria. They show a high pH-optimum, need ammonia or an amine as activator and are dimers of identical subunits of a molecular mass of 60 000. The dimer is the catalytically active form. Each subunit carries one prosthetic group pyrroloquinoline quinone, which can be titrated by the suicide substrate cyclopropanone ethylhemiketal. In contrast to the general methanol dehydrogenases the two ethanol dehydrogenases have a low affinity for methanol and in addition to primary alcohols they also oxidize secondary alcohols. With secondary alcohols preferentially one of the two enantiomers is oxidized.

The catalytic and spectral properties of the two enzymes are very similar to the quinoprotein ethanol dehydrogenase isolated from *P. aeruginosa* LMD 80.53 (Groen et al., 1984. Biochem. J. 223: 921-924). However this enzyme is reported to be a monomer of molecular mass 100 000.

INTRODUCTION

Methanol oxidizing bacteria possess dye-dependent alcohol dehydrogenases which contain pyrroloquinoline quinone as prosthetic group (Duine et al., 1980). The prosthetic group is also called methoxatin (Salisbury et al., 1979). The usual function of this alcohol dehydrogenase is to catalyze the oxidation of methanol. The isolated enzyme however oxidizes a wide range of primary alcohols, while secondary alcohols are not accepted as substrate. The pH-optima are around pH 9 and the $K_m$ values for methanol are in the range of 10 to 20 μM. The methanol dehydrogenases need ammonia or methylamine as activator and phenazine methosulfate is used as electron acceptor. In general the methanol dehydrogenases are dimers of identical subunits with a molecular mass of 60 000.
From *P. aeruginosa* LMD 80.53 grown on ethanol recently a quinoprotein alcohol dehydrogenase was purified, which has a low affinity for methanol and oxidizes in addition to primary alcohols also secondary alcohols (Groen et al., 1984). With respect to the spectral and catalytic properties this ethanol dehydrogenase is very similar to the usual methanol dehydrogenases. Recently we found that *P. aeruginosa* ATCC 17 933 produces high levels of such a quinoprotein ethanol dehydrogenase. This enzyme was purified to homogeneity and crystallized (Rupp and Görisch, 1988). In addition we purified and crystallized a quinoprotein ethanol dehydrogenase from *P. putida* ATCC 17 421, grown on ethanol. Both enzymes show catalytic properties similar to the ethanol dehydrogenase described for *P. aeruginosa* LMD 80.53 (Groen et al., 1984).

**MATERIALS AND METHODS**

**Chemicals.** CM-Sepharose was obtained from Pharmacia (Uppsala, Sweden). All other chemicals were obtained as described recently (Rupp and Görisch, 1988).

**Organisms and growth conditions.** *P. putida* ATCC 17 421 and *P. aeruginosa* ATCC 17 933 were grown on a mineral medium supplemented with 0.5% ethanol as described (Rupp and Görisch, 1988).

**Enzyme isolation.** 10 g of frozen cells of *P. putida* 17 421 were suspended in 12 ml of 50 mM Tris/HCl buffer, pH 7.9, and cells were disrupted by ultrasonic treatment. Cell debris was removed by centrifugation at 60 000 x g for 60 min at 4°C. All subsequent steps were performed at 4°C. The crude extract was applied to a DEAE-Sephascel column (3 x 13 cm) equilibrated with 10 mM Tris/HCl buffer, pH 7.9, and quinoprotein ethanol dehydrogenase was eluted by the same buffer. The active fractions were pooled and applied to a CM-Sepharose column equilibrated with 10 mM ammonium acetate/acetate buffer, pH 6.5. The enzyme was eluted by a linear gradient from 0 to 1 M NaCl in 10 mM ammonium acetate/acetate buffer, pH 6.5. The active fractions were concentrated by an Amicon YM 10 membrane and applied to a Sephacryl S-200 column (2.6 x 100 cm). The column was equilibrated with 100 mM Tris/HCl buffer, pH 7, containing 100 mM NaCl, and quinoprotein ethanol dehydrogenase from *P. putida* ATCC 17 421 was eluted by the same buffer. Active fractions were pooled and stored at -80°C.

Quinoprotein ethanol dehydrogenase from *P. aeruginosa* ATCC 17 933 was purified as described previously (Rupp and Görisch, 1988).

**Enzyme assay.** The activity of quinoprotein ethanol dehydrogenase was measured spectrophotometrically by following the reduction of either phenazine methosulfate/2,6-dichlorophenolindophenol (PMS/DCPIP) or Wurster’s blue as described (Rupp and Görisch, 1988). One unit of enzyme activity is defined as that amount, either reducing 1 μmol of DCPIP or 2 μmol of Wurst-